Tucson Water-
University of Arizona
Project Updates

March 25, 2015

Kelly A. Reynolds, MSPH, PhD, Associate Professor, Director
Jonathan D. Sexton, PhD, Research Specialist, K-12 Outreach
Robert Canales, PhD, Assistant Professor

Environment, Exposure Science and Risk Assessment Center (ESRAC)
Mel & Enid Zuckerman College of Public Health
The University of Arizona, Tucson, AZ

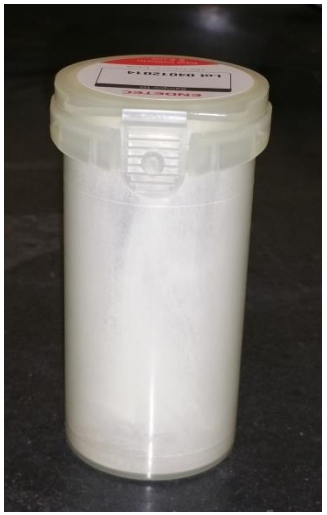
Endetec Validation

Kelly A. Reynolds, PhD, Associate Professor, Jonathan D. Sexton, PhD, Research Specialist, K-12 Outreach

Endetec

Pros

- Results in 18 hours or less
- Mechanically read
- Notifications of results
- Semi-quantitative



Cons

- Longer learning curve
- Sample number dependent on model (ie. 16 or 24)

Level of Severity

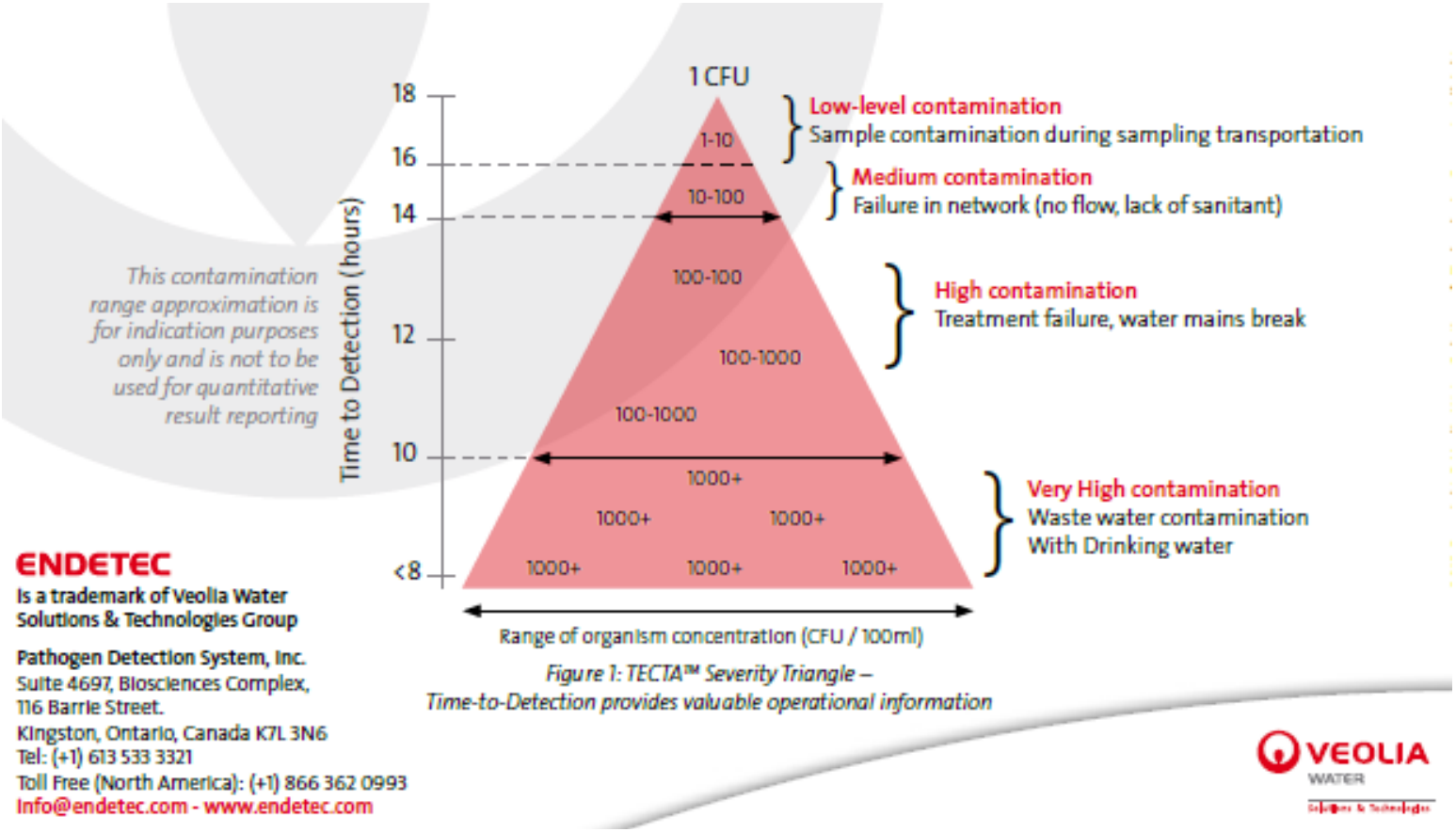


Figure 1: TECTA™ Severity Triangle –
Time-to-Detection provides valuable operational information

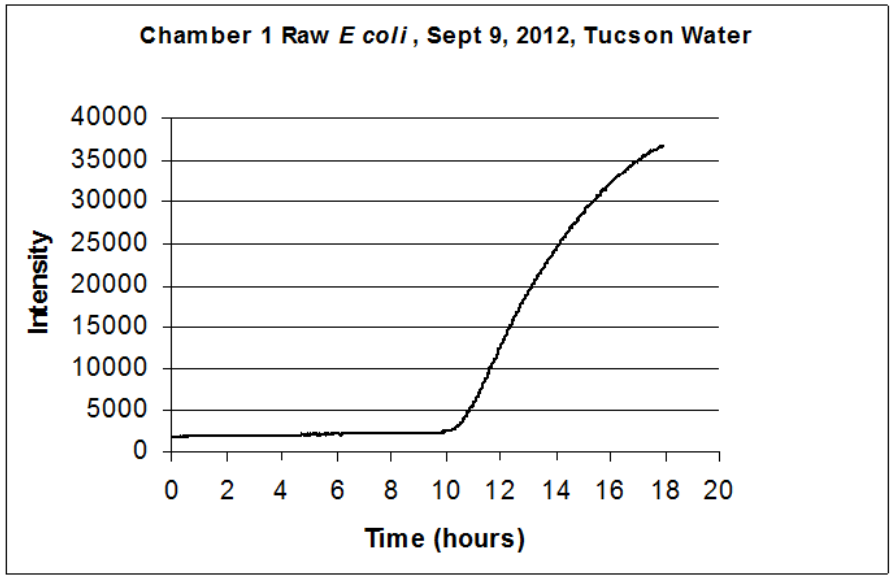
ENDETEC
Is a trademark of Veolia Water Solutions & Technologies Group

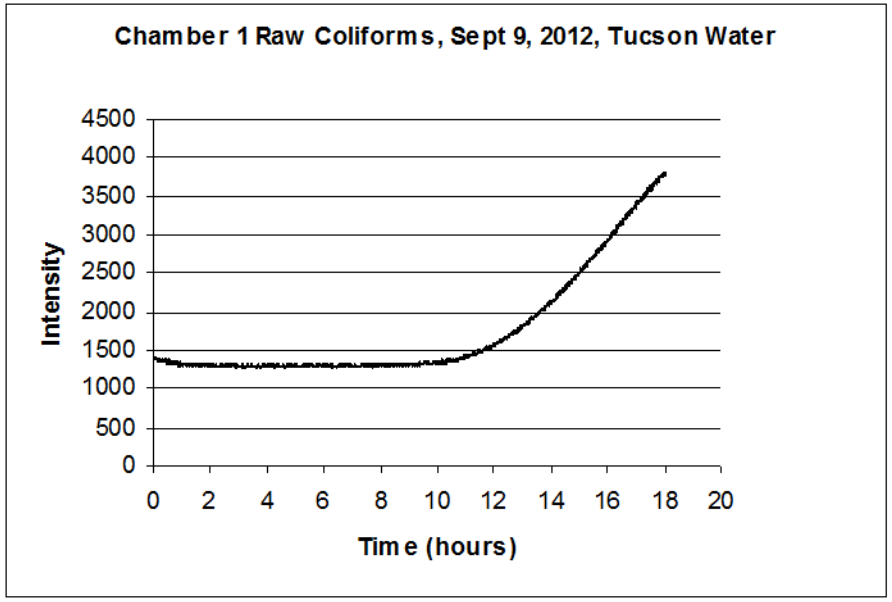
Pathogen Detection System, Inc.
Suite 4697, Biosciences Complex,
116 Barrie Street.
Kingston, Ontario, Canada K7L 3N6
Tel: (+1) 613 533 3321
Toll Free (North America): (+1) 866 362 0993
Info@endetec.com - www.endetec.com

VEOLIA WATER

Seeded Water

- Water was seeded with *Escherichia coli* and *Serratia rubidaea*
- No discrepancies between methods for concentrations ranging 10-10^7 cfu/100mL
- Concentrations <10 resulted in mixed results
  - Likely due to difficulties in diluting
- Level of severity was in the range of plate count concentrations


Chamber 1 Raw *E coli*, Sept 9, 2012, Tucson Water
Intensity
40000
35000
30000
25000
20000
15000
10000
5000
0
0 2 4 6 8 10 12 14 16 18 20
Time (hours)


Chamber 1 Raw Coliforms, Sept 9, 2012, Tucson Water
Intensity
4500
4000
3500
3000
2500
2000
1500
1000
500
0
0 2 4 6 8 10 12 14 16 18 20
Time (hours)

Drinking Water

- 150 drinking water samples collected in the Tucson area
- All negative for total coliforms except 1 sample
  - Low level of severity
  - No discrepancies between methods

Reclaimed Water

- 18 reclaimed water samples were collected
- 66.7% (12/18) positive for total coliforms
  - No discrepancies between methods
  - Low-Medium level of severity

Software update 2.0.4

- Improved user interface
  - Decreased learning curve
- Improved quantitative abilities

Quantitative Validation

- *E. coli* only

Endetec *E. coli* Concentration (cfu/100mL)	Plate Count Concentration (cfu/100mL)	Endetec Total Coliform Concentration (cfu/100mL)	Plate Count Concentration (cfu/100mL)
10^8	2.76E+09	10^9	2.76E+09
10^7	2.85E+08	10^8	2.85E+08
10^6	2.72E+07	10^6	2.72E+07
10^5	2.52E+06	10^6	2.52E+06
10^4	2.26E+05	10^5	2.26E+05
3.07E+03	2.90E+04	10^4	2.90E+04
238	2.70E+03	2.38E+03	2.70E+03
36	305	320	305

Quantitative Validation

- *S. rubidea* only

Endetec Total Coliform Concentration (cfu/100mL)	Plate Count Concentration (cfu/100mL)
10^7	4.55E+09
10^6	4.75E+08
10^4	4.85E+07
1.37E+03	4.20E+06
330	5.15E+05

Quantitative Validation

- *E. coli* and *S. rubidea*

Endetec *E. coli* Concentration (cfu/100mL)	*E. coli* Plate Count Concentration (cfu/100mL)	Endetec Total Coliform Concentration (cfu/100mL)	Total Coliform Plate Count Concentration (cfu/100mL)
10^8	2.55E+09	10^9	5.95E+09
10^7	2.85E+08	10^8	6.70E+08
10^6	1.90E+07	10^6	5.80E+07
10^5	2.35E+06	10^6	6.30E+06
10^4	2.50E+05	10^5	6.00E+05
6.55E+03	2.80E+04	10^4	7.60E+04
515	2.35E+03	9.13E+03	8.45E+03
53	400	317	1.00E+03
5	27	77	75
1	2	7	6

Future Work

- Comparison of methods with water of varying quality
  - Microbial and chemical
- Quantitative validation with different bacteria
  - Coliforms and non-coliforms

Tucson Water
Risk Assessment Project

Robert Canales, PhD, Assistant Professor
Kelly A. Reynolds, PhD, Associate Professor
Sally Littau, BS, MT(ASCP) Health Research Coordinator

Environment, Exposure Science and Risk Assessment Center (ESRAC)
Mel & Enid Zuckerman College of Public Health
The University of Arizona, Tucson, AZ

Brief Fluoride Summary

- Fluoride analysis has replaced the Chromium-6/Total Chromium analysis due to insufficient data currently collected
- 2 recent news reports suggested Tucson water is low in fluoride concentrations
  - Arizona Daily Star Nov 2, 2014
  - Arizona Daily Wildcat, Nov 11, 2014

Fluoride Benefits and Risks

- At low intake levels, fluoride has can have therapeutic value in the prevention of dental caries
- Slightly higher levels can lead to dental fluorosis - a condition in which the enamel covering of the teeth fails to crystallize properly
  - More of a concern for children during the period of enamel development
  - Possible problems range from barely discernible markings to brown stains and surface pitting
  - Some studies show that climate may be a factor as well
- Prolonged high intake can result in skeletal fluorosis - a condition which may increase bone brittleness and risk of bone fracture
- In high-dose cases, severe bone abnormalities can develop

Current Fluoride Guidelines

–Regulated by EPA

- Maximum contaminant level goal (MCLG) is 4.0 mg/L (4.0 ppm)
- Enforceable MCL is 4.0 mg/L
- Non enforceable secondary level of 2 mg/L

American Dental Association

- ADA recommendation
  - Optimum water fluoride concentration of 0.7 to 1.2 ppm
  - Was established to maximize the decay preventive benefit
  - 2014 article by American Academy of Pediatric Dentistry “Guideline on Fluoride Therapy”
    - Department of Health and Human Services
    - Recently proposed 0.7 as the upper limit due to additional sources of fluoride available (toothpaste, for example)

Agency for Toxic Substances and Diseases

- ASTDR minimal risk level for sodium fluoride
  - Oral Route: 0.6 mg/kg/day
  - Endpoint: Musculoskeletal (fluorosis, skeletal fracture)
- Other potential risks:
  - High levels: Cancer
  - Low levels: Dental caries

Questions

- Currently the level of fluoride in Tucson water fluctuates across sampling points and over time

- Does Tucson have plans to fluoridate water?
- Is there anticipation of public concern?
- What are major concerns and considerations?

Data Files

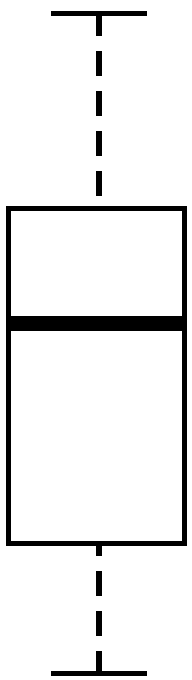
- Received 71 data files from TW
  - 60 are WQZ files; 11 from sites such as Sunset Ranch, Diamond Bell, Thunderhead etc.
  - May enable us to provide displays or data summaries for data combined over all years
- Contains all laboratory data for
  - Years 2009 to 2014
  - All Tucson water systems
  - Each WQZ file has data for 10 – 36 sampling points

File Formatting

- Original XML files were archived and copied, and copied files were converted to text (.txt) files for easy upload into statistical software (R)
- Joined all years (2009 – 2014) for a given WQZ
- Used SAMPLE_DATE field to create 2 fields corresponding to year and month, for further temporal analysis
- Created new data frames extracting values relevant to analysis of Fluoride
- All commands for formatting and analysis are saved in scripts for quality assurance

Potential and Preliminary Analysis

- Use of boxplots (box and whisker plots) to visualize data summaries



maximum

75th percentile

median

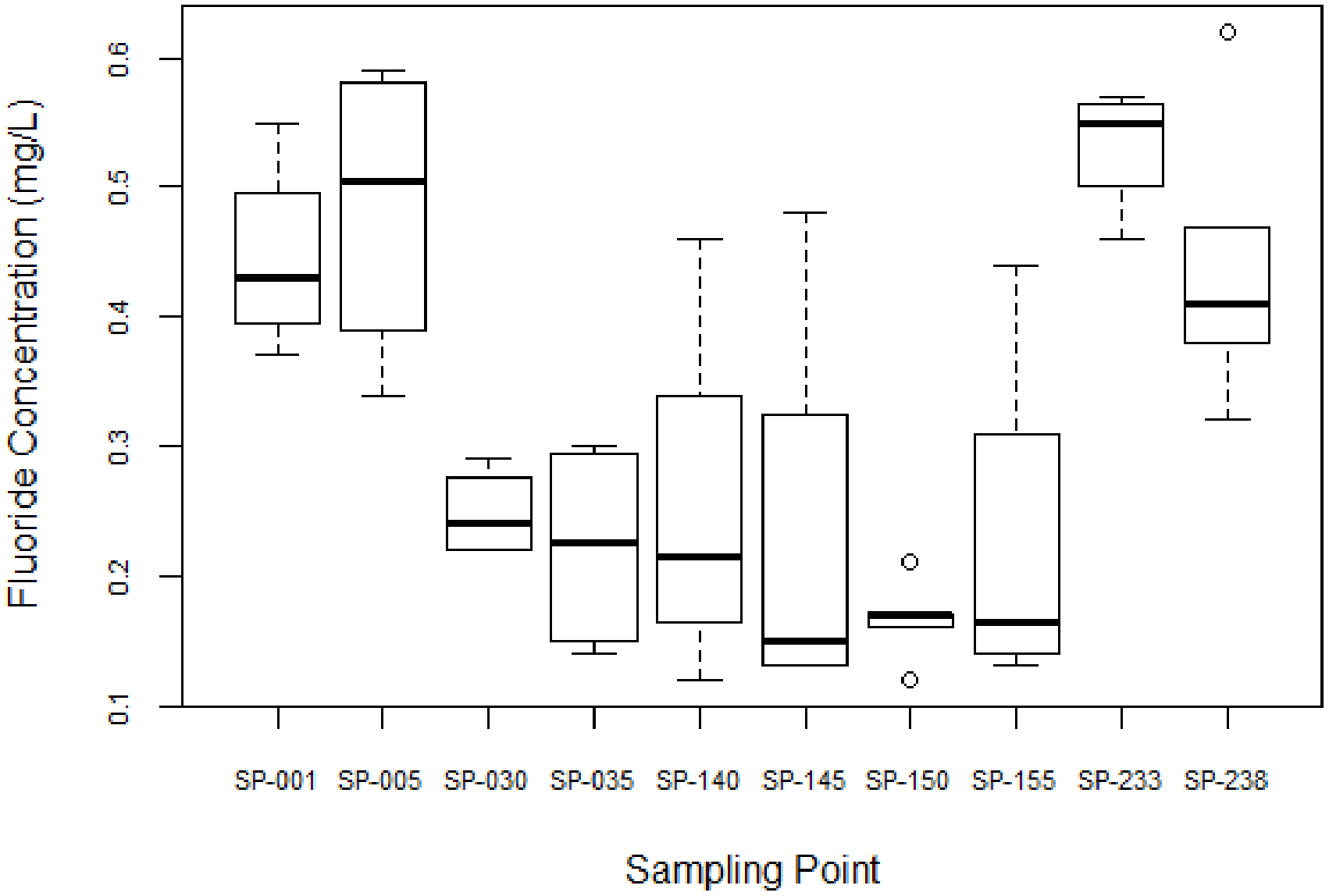
25th percentile

minimum

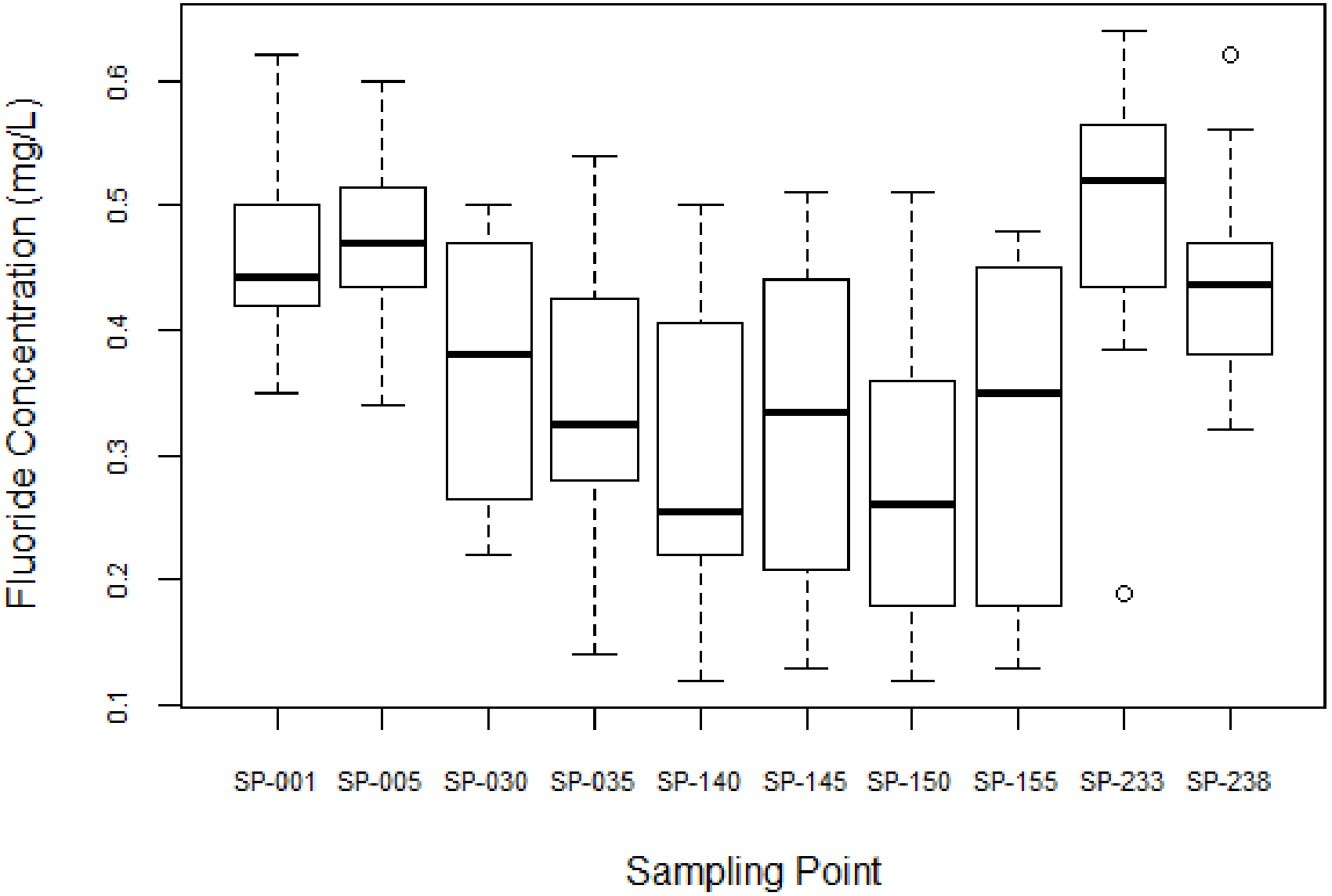
Any outliers are indicated as points beyond the “whiskers”

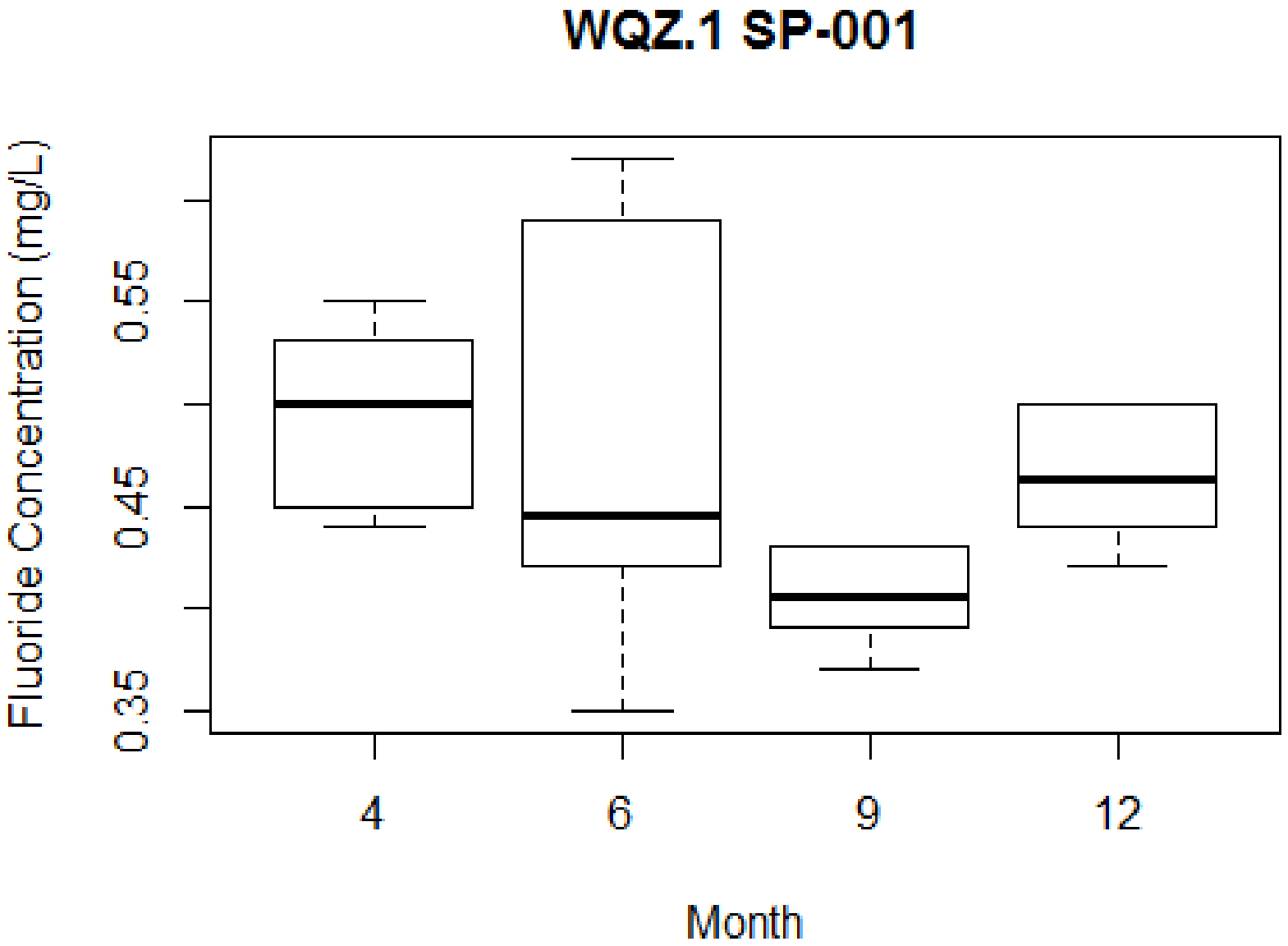
Potential and Preliminary Analysis

- Use of boxplots
  - Visualize data from each sampling point across a water zone for a particular year
  - Visualize data from each sampling point across a water zone for the years 2009 – 2014 combined
  - Visualize data from a sampling point for all years by sampling date (month and/or season)


WQZ.1_2009
Fluoride Concentration (mg/L)
0.1
0.2
0.3
0.4
0.5
0.6
SP-001
SP-005
SP-030
SP-035
SP-140
SP-145
SP-150
SP-155
SP-233
SP-238
Sampling Point

WQZ.1 2009-2014




WQZ.1 SP-001
Fluoride Concentration (mg/L)
0.35
0.45
0.55
4
6
9
12
Month

Future Work

- Continue exploration along the lines of sample plots, data summaries, and trends
- Explore correlations between Fluoride and metals or other water components/characteristics
- If given coordinates, can map data along sampling points across water zones

Future Work

- Indirectly estimate fluoride dose intake via drinking tap water
  - Use intake data from EPA handbooks or consumption surveys
- Use additional data sources and assumptions to estimate cumulative exposure assessment and dose from multiple sources
- Estimate health risks from tap water and cumulative sources
  - Use of EPA's dose-response information

Statistical Significance

- "What is the minimum amount of values, in general, that are needed to make the risk assessment model statistically significant for any given parameter?"

- What is the specific statistical test of interest?
  - Do we want to test if concentration values are statistically different from the MCLG of 4.0 mg/L?
  - What is the expected standard deviation or variance?
  - What is the expected effect size or difference in means?

Statistical Significance

- Assumptions
  - Desired level of significance of 0.05
  - Aim to achieve 80% power
  - One-sample test (test data against a single value such as the MCL)

- Sample size estimate is
  - n ≈ 15 for a large effect size (0.8)
  - n ≈ 25 for a medium effect size (0.5)
  - n ≈ 200 for a small effect size (0.2)
  - Interpretation: to be able to detect a statistically significant difference that is small requires a greater sample size

Statistical Significance

- An effect size is the difference in means divided by the standard deviation
- Assuming a standard deviation of 0.1 mg/L (from preliminary analysis of data), the difference in means is
  - for a large effect size (0.8), 0.08
  - for a medium effect size (0.5), 0.05
  - for a small effect size (0.2), 0.02

- If testing the difference from the MCLG of 4.0 mg/L, based on preliminary analysis the expected effect size is likely large, requiring a relatively small sample size (~15) to achieve 80% power

SMARTPHONE FOR WATER QUALITY:

Smartphone Detection from Paper Microfluidics for Monitoring Water Safety

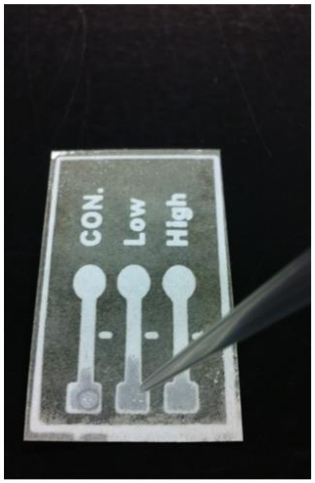
Jeong-Yeol Yoon, PhD, Associate Professor
Kelly A. Reynolds, MSPH, PhD, Associate Professor

Department of Agricultural & Biosystems Engineering (Yoon)
Mel & Enid Zuckerman College of Public Health (Reynolds)
The University of Arizona, Tucson, AZ

The Idea

Taking reference data

or



Loading sample on the paper microfluidic chip by dipping or pipetting

Taking signal data

Detection @ optmum angle utilizing internal gyro sensor

Scatter from paper is minimized utilizing Mie scatter theory

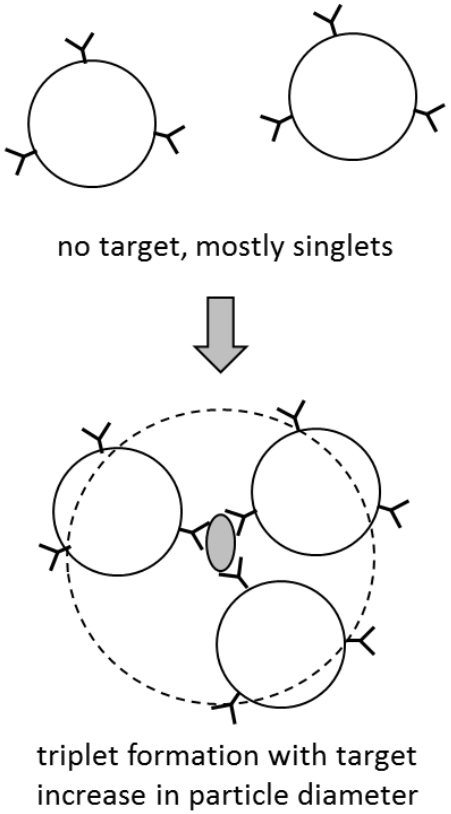
Filtration by paper fibers

Innovation

- Both paper microfluidics and smartphone-based biosensor have not been utilized for water quality monitoring (especially for pathogens).
- Method has demonstrated extremely low detection limit (10 pg virus antigens or 10 CFU bacteria per mL sample).

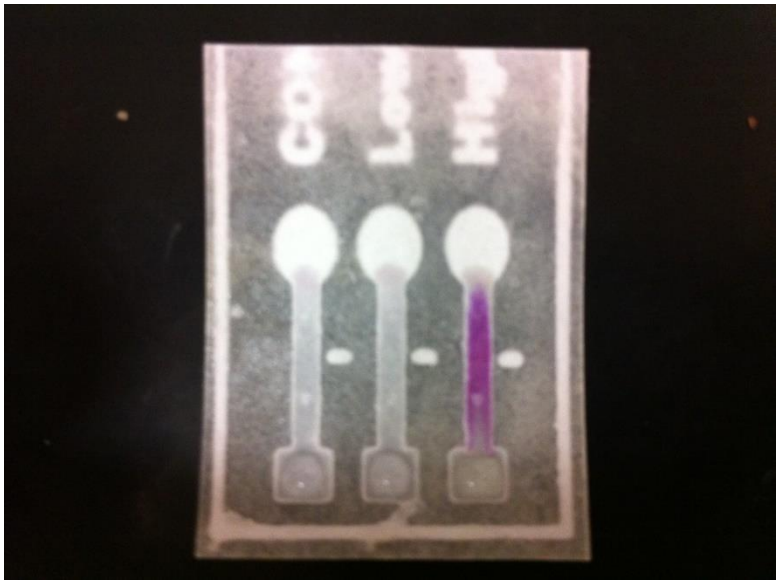
How it works

FOR PATHOGENS:



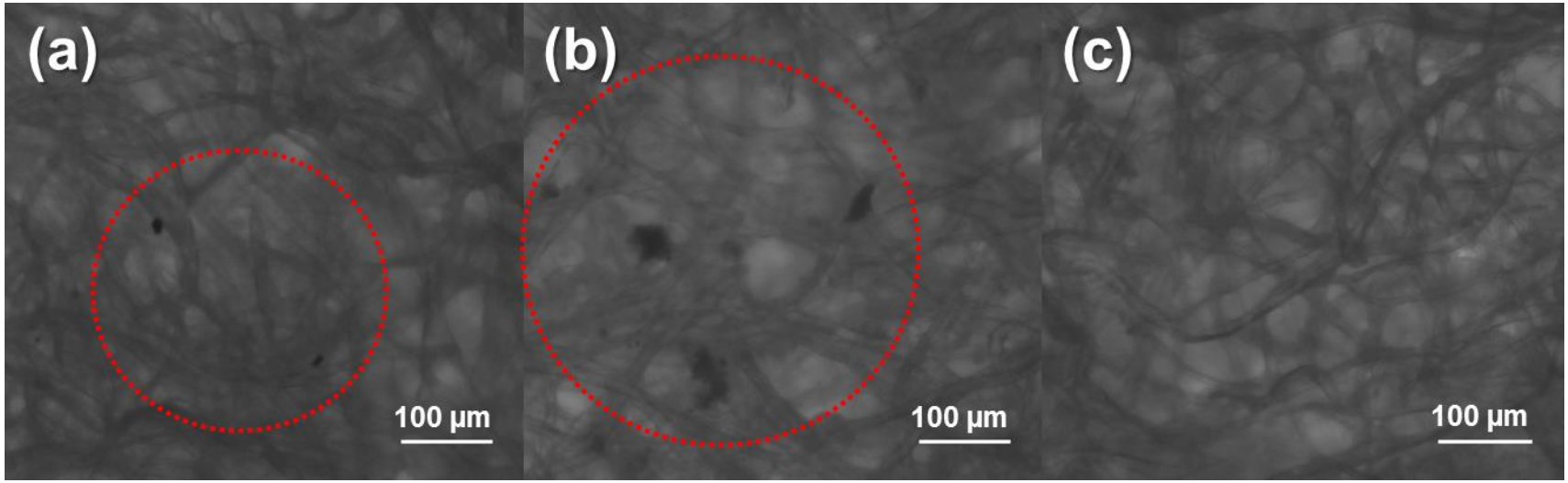
Detection by Mie scatter
@ optimized angle

FOR CHEMICALS:



Colorimetric assay
using RGB pixel intensities
with double normalization
(to cancel out chip-to-chip variation
and ambient lighting)

Filtration by paper fiber

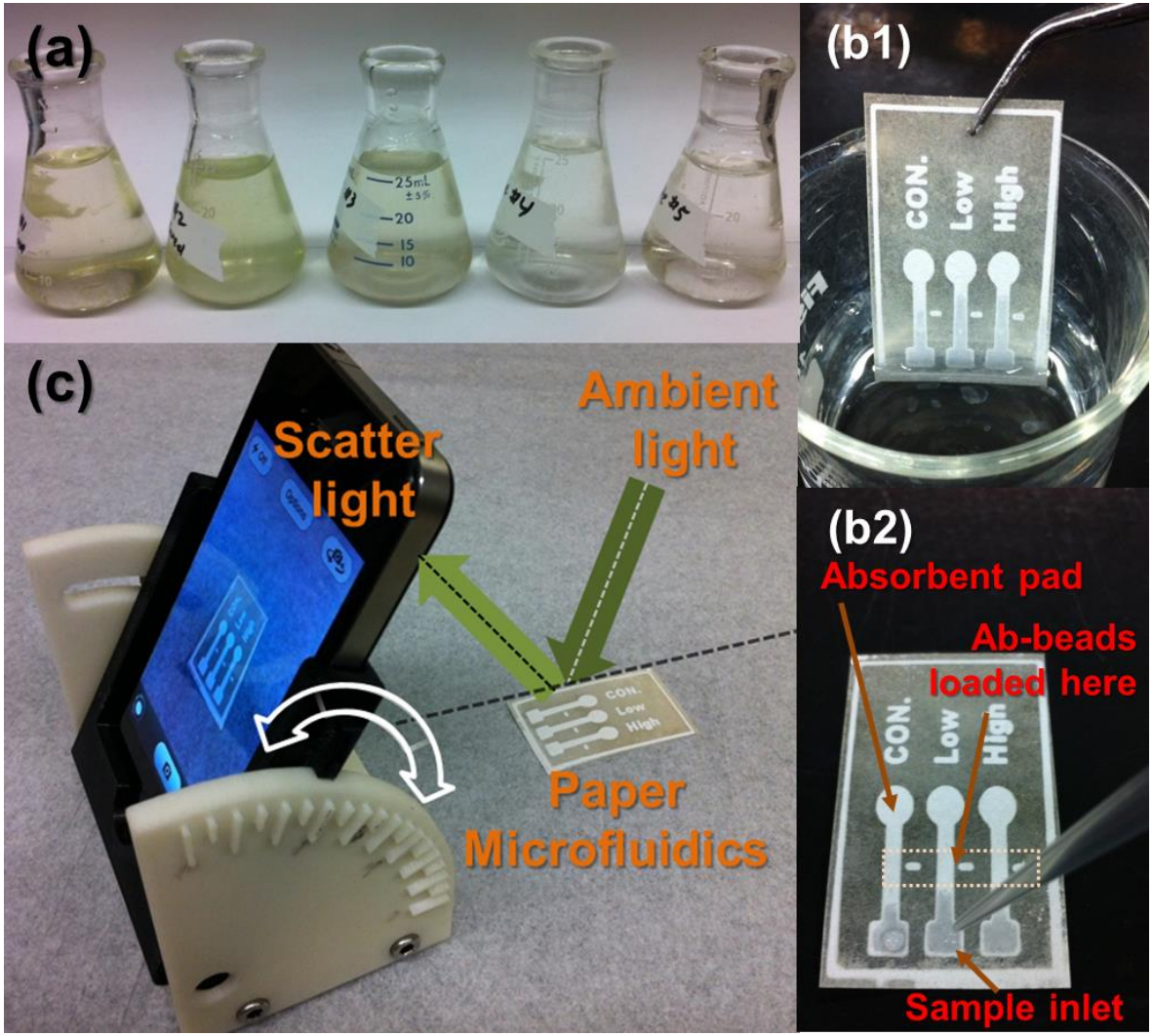


Soil particles
@ inlet

Algae
@ inlet

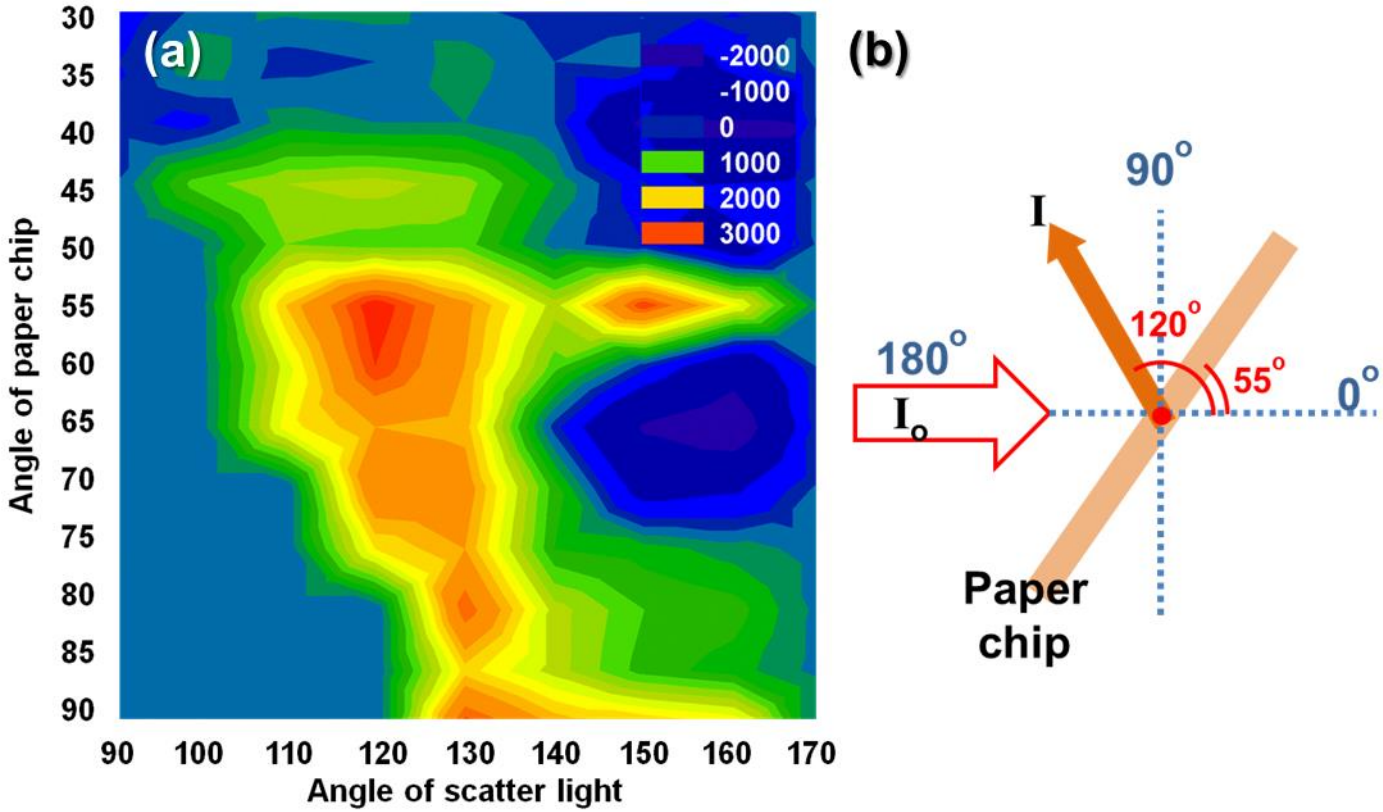
No contaminants
@ channel

Smartphone + paper microfluidics

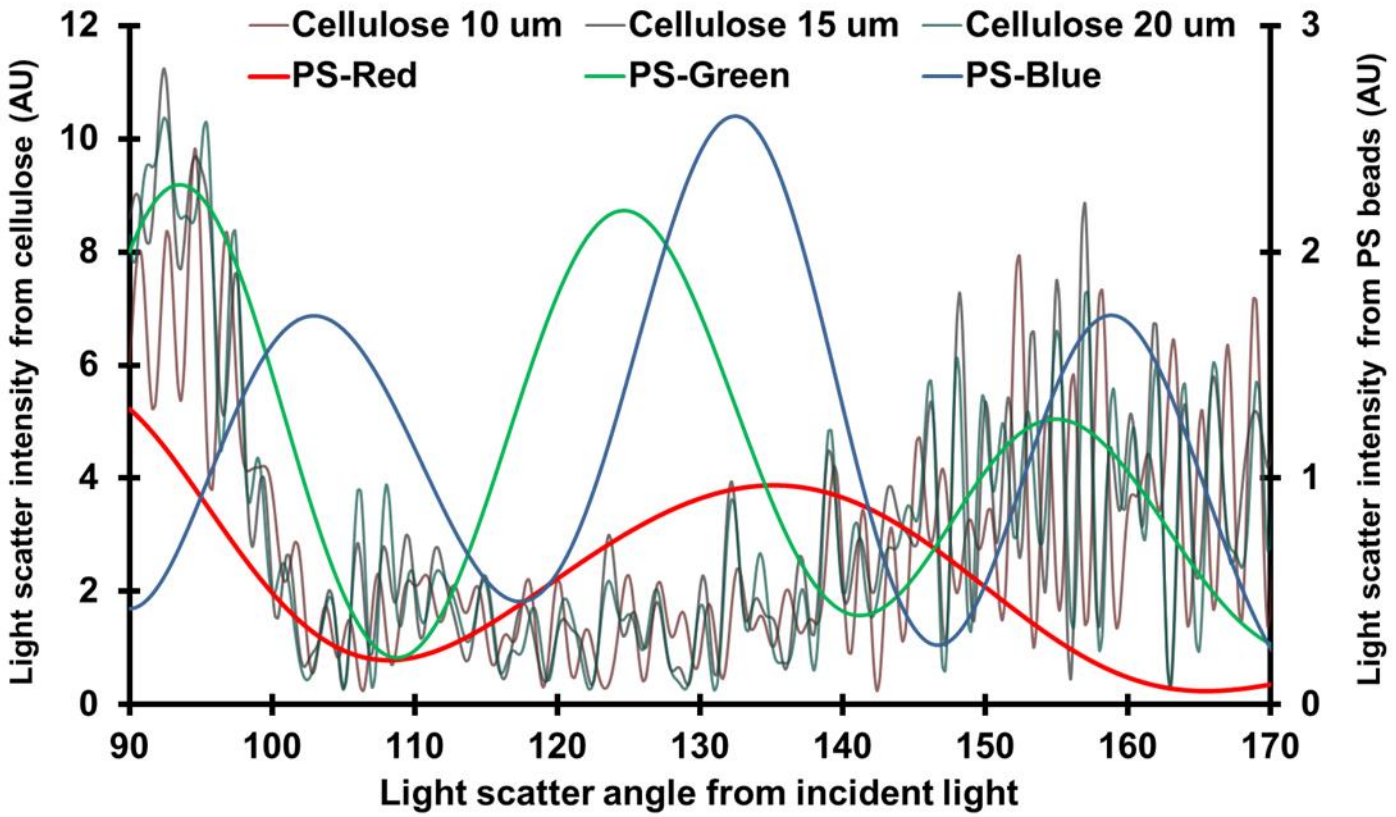


Park, Li, McCracken & Yoon, Lab Chip 13: 4832-4840 (2013)

Optimization of detection angle

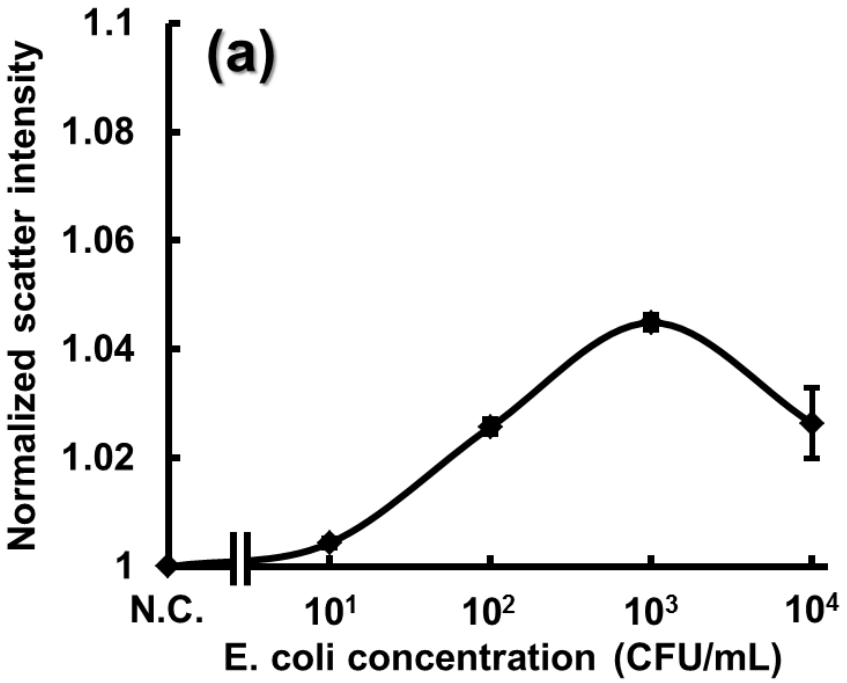


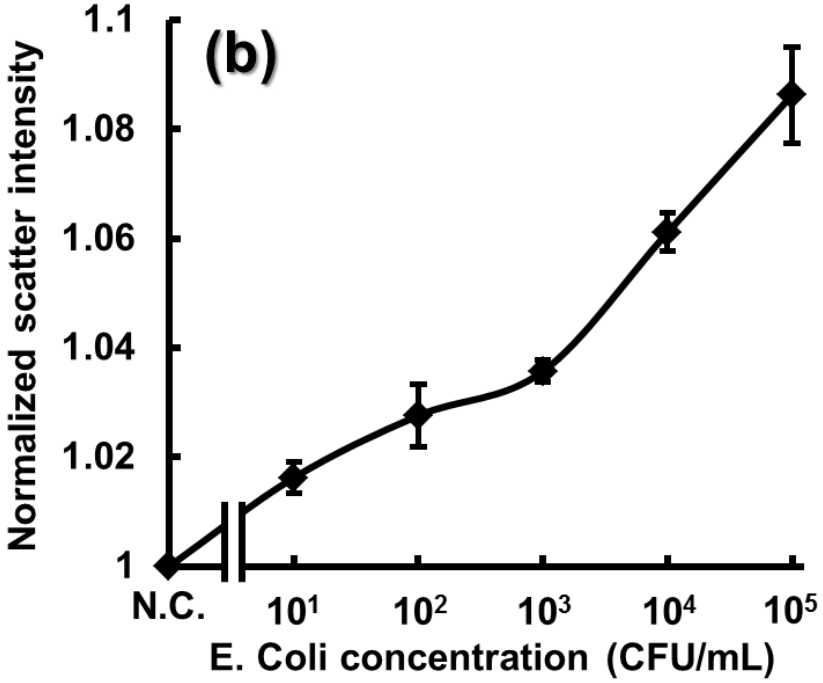
Mie scatter simulation



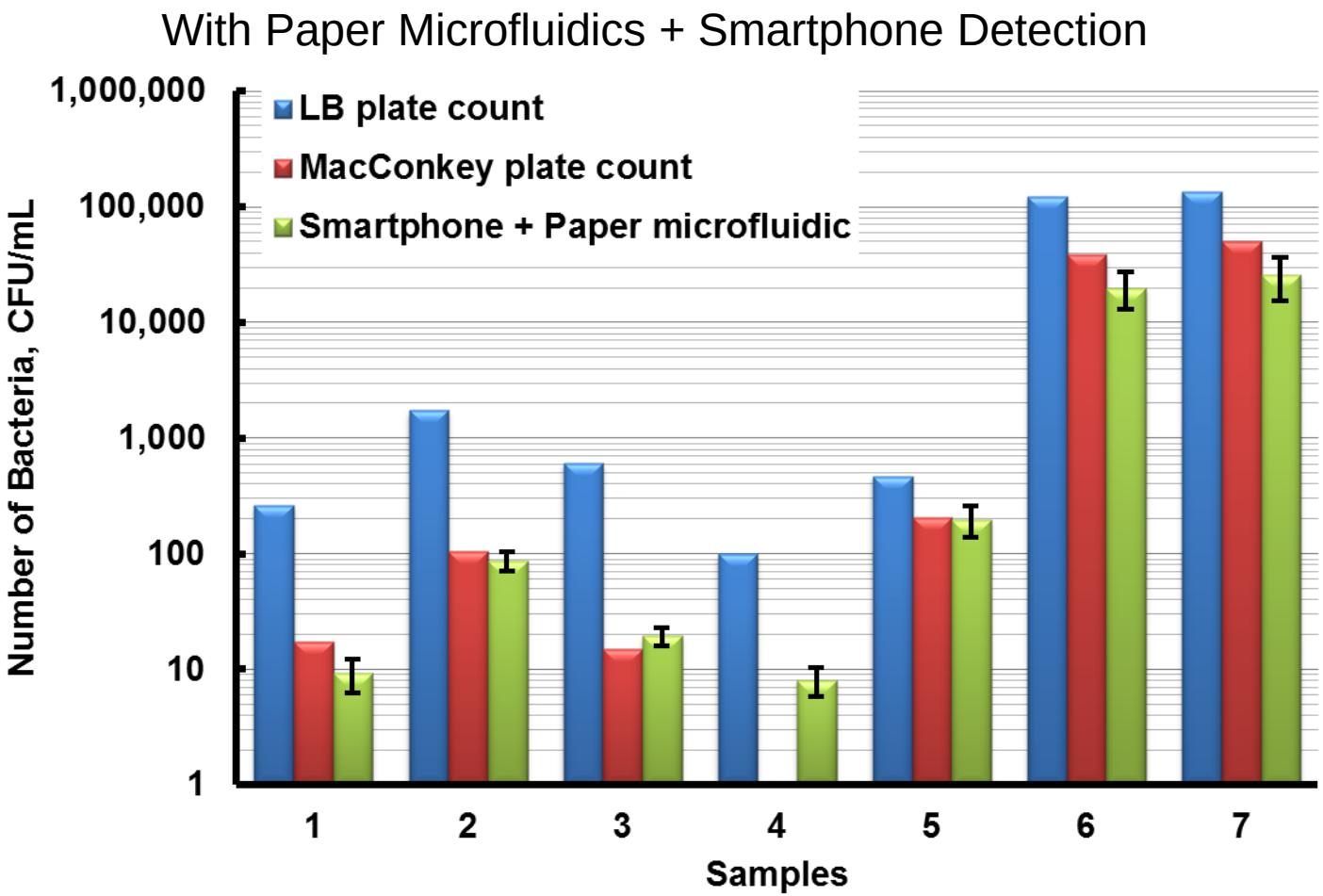
Standard curves

With Paper Microfluidics + Smartphone Detection



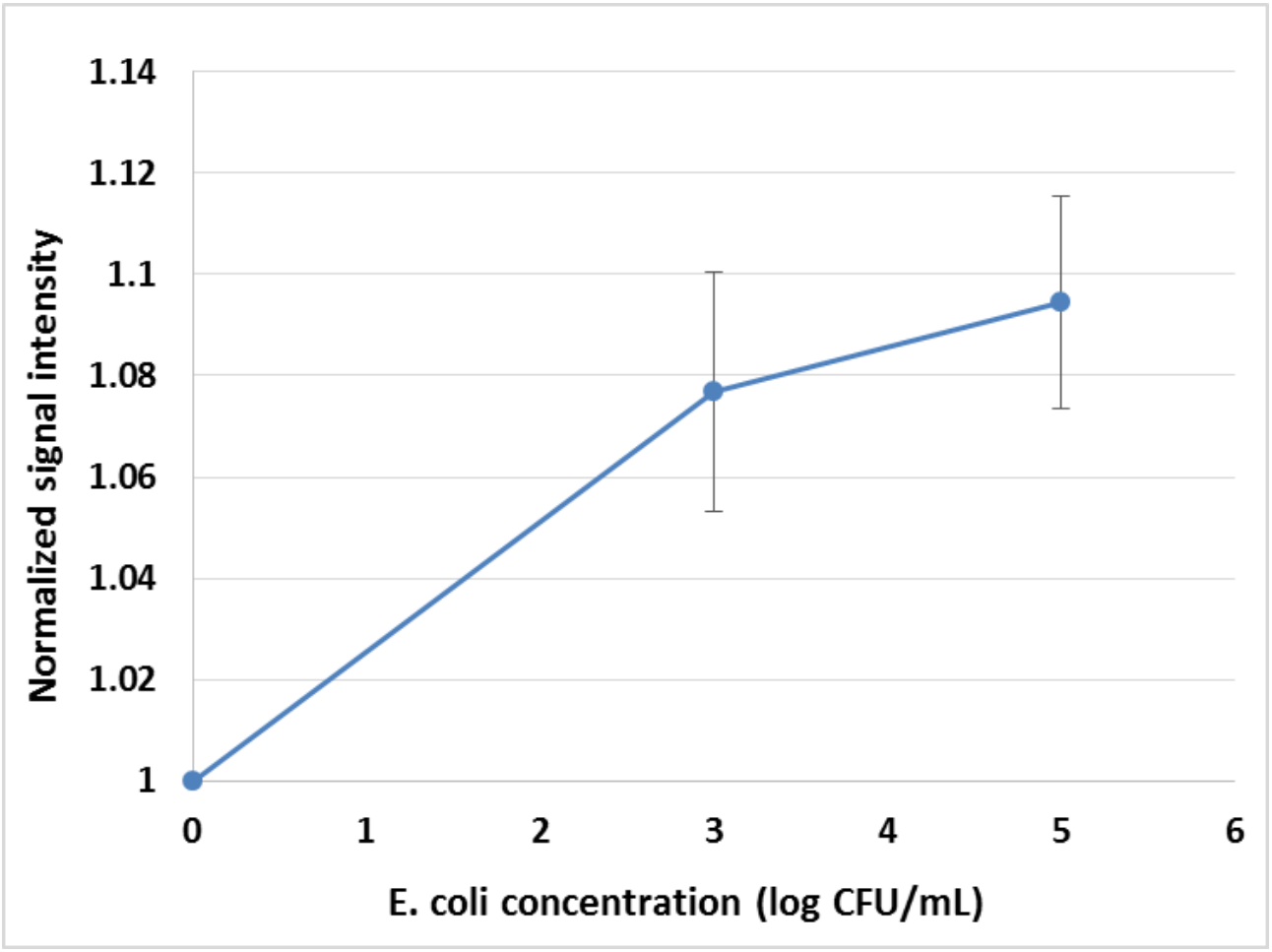


Field water samples



Results w/ 1.5 ppm chlorine

With Paper Microfluidics + Smartphone Detection

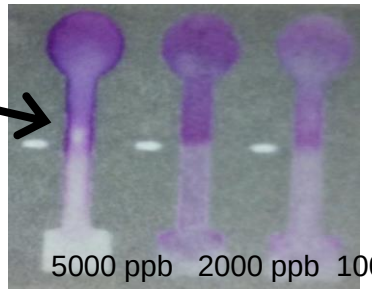


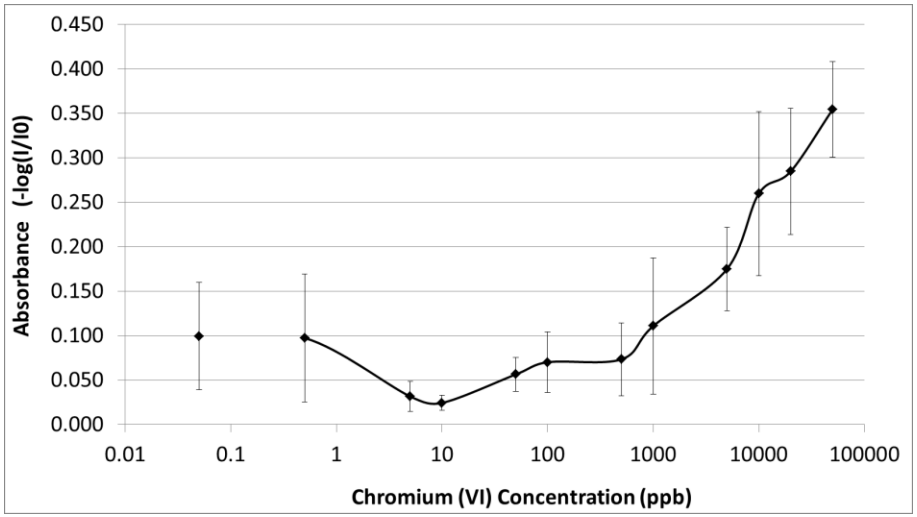
Able to detect *E. coli* in the presence of chlorine

Chromium (VI) and Chlorine Detection

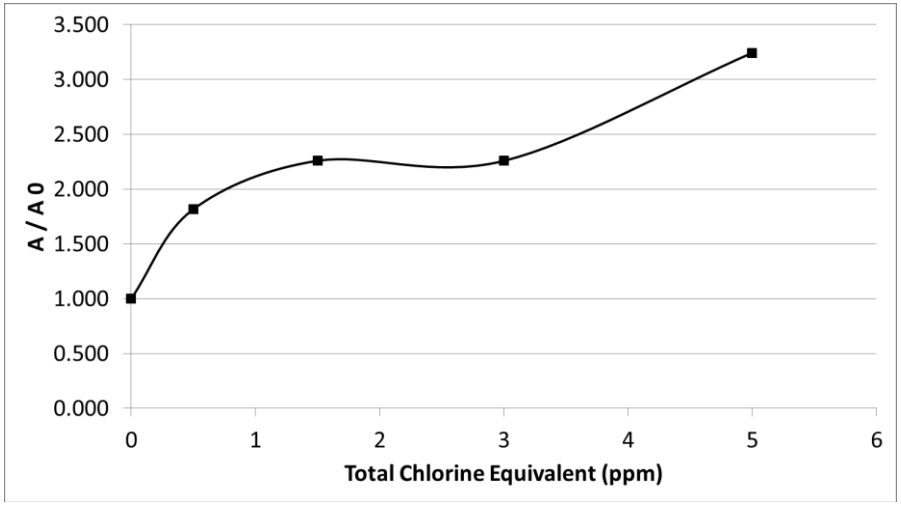
Dye Loaded In Channel Before Sample

Sample Loaded & Flows to Dye



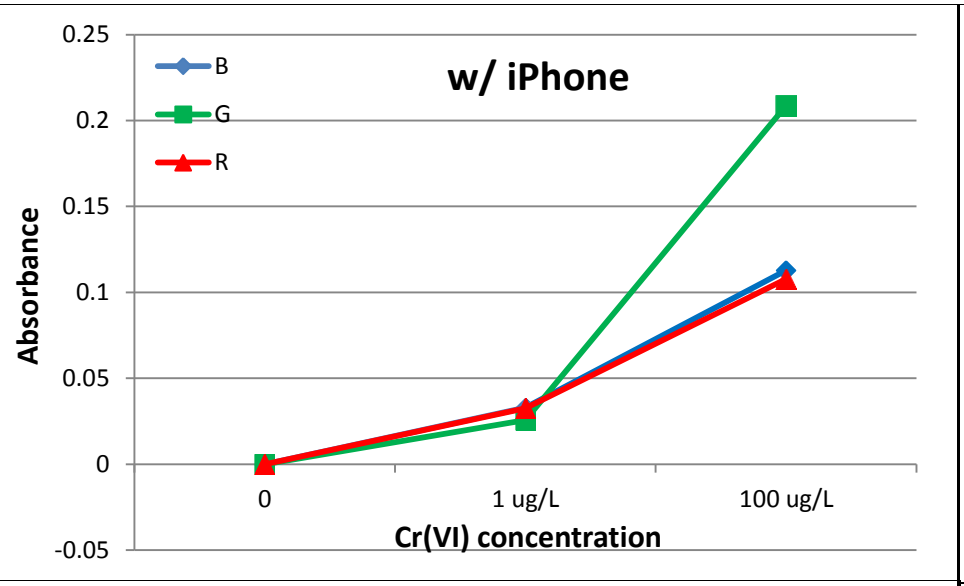


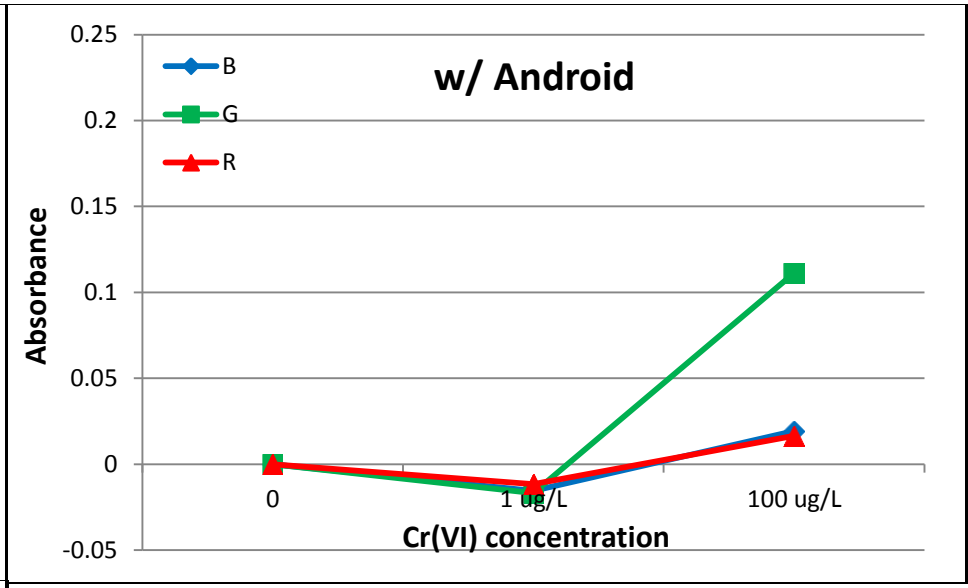
Chromium (VI) concentrations were quantified through green absorbance following a dying process mid-channel with diphenyl-carbazide (DPC)/H2SO4.

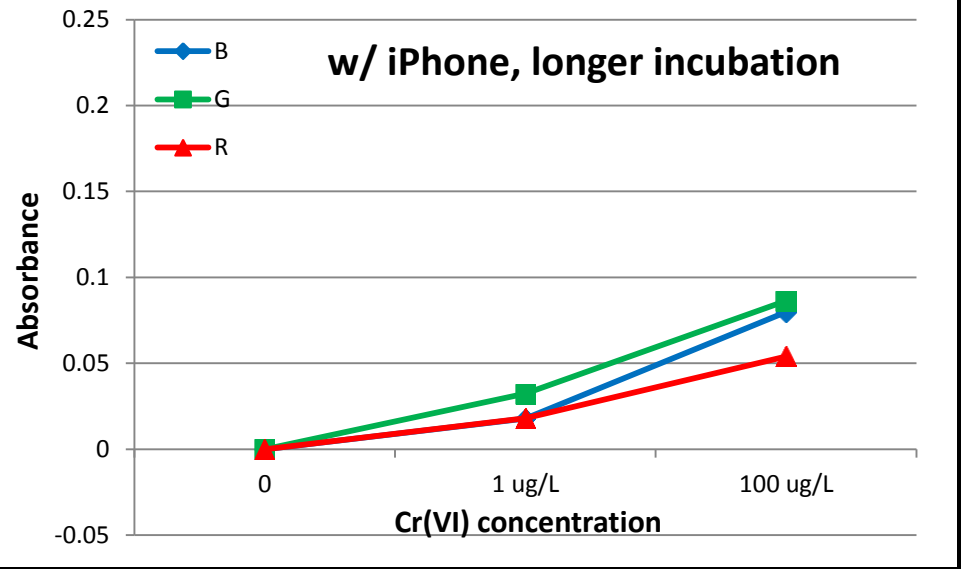


Chlorine quantified through green absorbance following N,N-diethyl-p-phenylenediamine (DPD) dying.

Preliminary result for cr(vi)







Detection limit of EPA Method 7196A w/ spectrophotometer = 10-20 ug/L

This method = ca. 1 ug/L

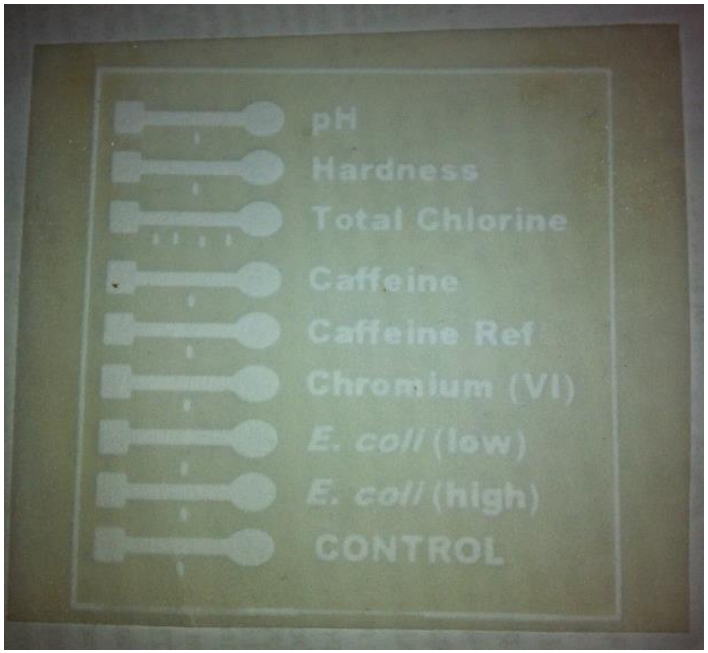
Conclusions

- Paper microfluidics with smartphone detection permits rapid and sensitive water quality detection at environmentally significant levels for customizable targets
  - **Single-cell *E. coli*** detection (assay < 90s)
  - **10 ppb Chromium (VI)** detection (< 10 min)
  - **0.5 ppm Total Chlorine** detection (< 10 min)
- Smartphone-based assay allows mobility for potential in-field, real-time detection
- Technology advancing
  - Improved LED flash technology and smartphone camera resolution
  - Improved app, autosearches optimal light scattering angle

Multi-channel paper microfluidics

Detection of water quality parameters with paper microfluidics:

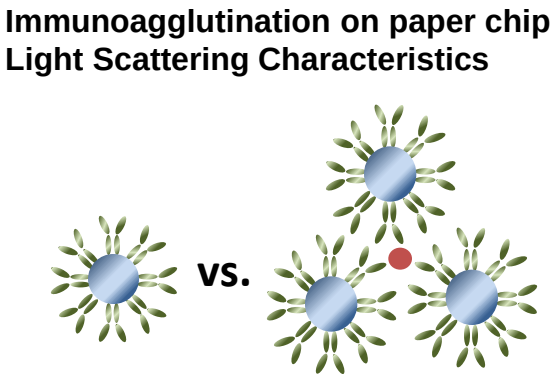
pH
Total Chlorine
Hardness
    Ca^{2+} and Mg^{2+}
E. coli
Chromium (VI)
Caffeine

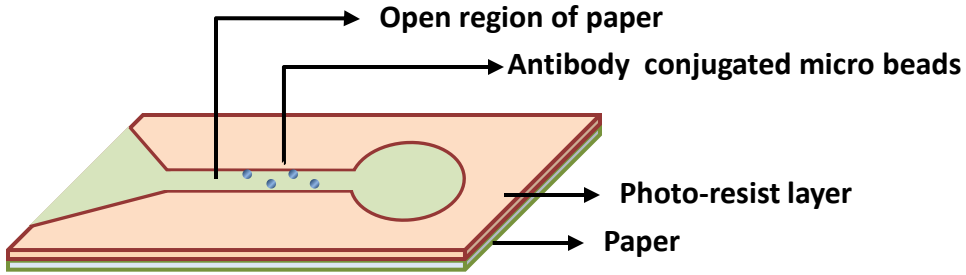


Future work

- Repeat assays of *E. coli* in complex water samples (reuse water)
- Comparison with routine Colilert® monitoring
- Combined microbe detection
- Colorimetric assays for other parameters, including arsenic and dioxin
- Advance virus detection method

Paper Microfluidics:
Particle-based Immunoassay for norovirus





Using antibodies on paper to detect norovirus capsid protein VP1

Experiments to be conducted with recombinant norovirus antigen, and deactivated norovirus capsid (both from identified sources)

Acknowledgements

- **Tucson Water**
  - Dan Quintanar
- **NSF-WET Center**
  - Dr. Ian Pepper

- **Environment, Exposure Science and Risk Assessment Center (ESRAC)**
  - Dr. Robert Canales
  - Dr. Jonathan Sexton
  - Melissa Valdez, MSPH
  - Sally Littau, BS, MT, (ASCP)
- **Biosensors Lab, University of Arizona**
  - Dr. Jeong-Yeol Yoon
  - Dr. Tu San Park
  - Dr. Scott Angus
  - Katherine McCracken